

Chinese Patent

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Title: Transgenic *Dunaliella salina* as a bioreactor

Abstract

Methods for preparing a transgenic *Dunaliella Salina* bioreactor, comprising introducing a foreign target gene into the cells of *Dunaliella Salina* and screening the transformed cells of *Dunaliella Salina*, are provided. The bioreactor of the present invention can be used as a safe and cheap production system for pharmaceutical proteins such as tumor necrosis factor (TNF), hepatitis B surface antigen (HBsAg).

Claims

What is claimed is:

1. A method for preparing a transgenic *Dunaliella Salina* bioreactor, comprising:
 - (a) constructing a heterologous expression vector containing a foreign target gene and a selectable marker;
 - (b) introducing the expression vector into the cells of *Dunaliella Salina* by using the methods for genetic transformation; and
 - (c) screening the transformants of *Dunaliella Salina*.
2. The method of claim 1, wherein said foreign target gene derived from humans and animals is at least one selected from the group consisting of tumor necrosis factor (TNF), hepatitis B surface antigen (HBsAg), etc.
3. The method of claim 1, wherein said selectable marker is at least one selected from the group consisting of spectinomycin or streptomycin resistance encoded by the aadA gene, herbicide phosphinothricin (PPT) resistance encoded by the BAR gene, etc.
4. The method of claim 1, wherein said techniques for introducing foreign target genes into the cells of *Dunaliella Salina* are one or more of the methods for genetic transformation selected from the group consisting of physical and/or chemical methods.
5. The method of claim 4, wherein said physical and chemical methods can be one or more of the group consisting of electroporation, gene-gun or PEG-mediated transformation.
6. The method of claim 2, further comprising the steps of constructing a *Dunaliella Salina* chloroplast expression vector p64C-TNF-MD containing the TNF gene or a *Dunaliella Salina* nuclear expression vector pCAMBIA-CtxB-SS1 containing the HBsAg gene before introducing